

XXIV.—On the Investigation of Vegetable Tissue by the aid of Polarized Light. By H. VON MOHL.

[Concluded from p. 209.]

A NEW and surprising series of phenomena is produced when the polarized pencil of light is made to pass through a doubly-refractive medium, for instance, a thin plate of gypsum, mica, or rock-crystal*, &c., in its way from the lower Nicol to the object to be investigated. If these plates are so placed that their neutral axes are directed obliquely to the Nicol (best at an angle of 45°), the field appears more or less illuminated according to the thickness of the plate used, and, if the plate has a certain thickness, to be determined by trials, the field assumes one of the colours of the Newtonian rings. The microscopic observation made by the help of this modified light is of a mixed kind, because the object is, on the one hand, seen in transmitted light as in the ordinary microscope; on the other hand, this light is on its own part again modified by the substance of the object, and in consequence of this the object appears, as in the cases above examined, as a self-luminous body, its various parts at the same time presenting bright tints of complementary colours according to their position in relation to the selenite-plate and to the Nicols. It is well known that this arrangement is made use of for the detection of weak degrees of doubly-refractive power, since many objects which do not reveal this power by mere application of two Nicol's prisms, are shown to be doubly-refractive bodies on the interposition of such a plate, by their peculiar colour differing from that of the field. It is not this arrangement, however, which I here wish to discuss, but the fact, hitherto overlooked, that there exist, in the behaviour of vegetable membranes to polarized light, variations analogous to those between positive and negative crystals, and that these variations stand in connexion with chemical distinctions in the vegetable organs.

This phenomenon may be observed either in annular structures or in transverse sections of smooth cell-membranes. Between the lower Nicol and the object is placed a plate of selenite, which renders the field red; this plate is so rotated that its neutral axes form an angle of 45° with the Nicols. If the microscope is now focussed to an annular object, for example, the cross-section of a cylindrical cell, this is seen divided into four quadrants, which appear tinged brightly with complementary colours in this way: the two alternate quadrants whose

* I shall give details respecting the choice and application of these plates in a future paper describing the arrangements of the polarizing microscope.

middle line corresponds to one of the neutral axes of the selenite-plate are either blue or green, the other two yellow or red. If the selenite-plate is rotated so far that its neutral axes are perpendicular to the Nicol, all the colours are lost, and they reappear on the continuation of the rotation, but in reverse order, the quadrants previously blue now appearing yellow, and *vice versâ*. This alternation is repeated at each quarter of a revolution.

When the object used is the transverse section of a cellular tissue with rectilinear side-walls, all the cell-walls which stand perpendicular to one of the Nicols will exhibit the colour of the field; all those which run parallel with one of the neutral axes of the selenite-plate, or form no great angle with it, will be blue, and those parallel with the other axis yellow. When we direct our attention to the concentric lamination of the annular cell-membrane, and the rectilinear of the membranes of polyhedral cellular tissue, we find that in both objects the same colour occurs in the lamellæ of the same direction.

But if we compare (of course without changing the position of the selenite-plate; keeping also constant attention to the identical direction of the lamination of the organ) vegetable elementary organs of various kinds, in reference to colour, we find that they fall into two classes, which are contrasted in regard to the colours which they exhibit under the given circumstances. In one class all the layers which lie obliquely in the direction of a right-wound screw, are coloured blue (or green), those lying in the direction of a left-wound screw-line, yellow (or red); in the second class the colours are opposite for the same directions; the organs of one class are optically positive, those of the other optically negative.

To the optically negative class belong the membranes of all elementary organs situated in the interior of a plant, whether they be left in their natural condition, or cellulose be purified from the infiltrated substances by the help of nitric acid and chlorate of potash. In this respect agree not only ordinary cells and vessels, but even structures whose substance is assumed by many chemists to be essentially different from cellulose, for instance the medullary cells of *Sambucus nigra*, the mucilaginous secondary layers of the hairs of the seeds of *Acanthodium spicatum*, the cells of Lichens and Fungi, collenchyma-cells, the horny endosperm of *Phytelephas*, the cells of the cotyledons of *Schotia speciosa*, the gelatinous cells of the Algæ, for instance of *Bangia atropurpurea*; finally, among the parts lying nearer to the surface, the fibrous cells of the envelope of the roots of Orchidæ and Aroideæ.

On the other hand, optically positive colours are exhibited by

the cell-membranes of the periderm and the cuticular layers of the epidermal cells. This occurs, for instance, in common cork, in the periderm of *Quercus Cerris*, *Prunus virginiana*, *Æsculus Hippocastanum*, *Betula alba*, and in the thin cork-layers which divide the bark of old trunks of *Pinus*, e. g. *P. nigricans*, into scales. In the epidermis all those membranes which are coloured blue by iodine and chloride of zinc, like an ordinary cell-membrane, behave in optical respects also like a cellulose membrane; while all those layers which are coloured brown by those reagents, like the cell-membranes of the periderm, are optically positive. It here makes no difference whether the cuticle forms but a thin pellicle on the outer surface of the epidermis, as in most thin-walled epidermal cells (e. g. on the rhizome of *Polypodium aureum*, in the stem of *Impatiens*, of *Euphorbia Caput Medusæ*, on the leaf of *Helleborus fœtidus*, &c.), or, as in thick-walled epidermal cells, a thick stratum of the lamellæ on the external wall of the epidermal cells, and parts of their side-walls, possess the property of cuticle,—as, for instance, in the leaves of *Cycas revoluta*, *Phormium tenax*, *Hakea gibbosa*, and *Aloe obliqua*, and in the stems of *Viscum album* and *Misodendron*.

This contrast between the cuticular layer and the parts of the cell-wall composed of unaltered cellulose, may be observed with extreme clearness in the epidermis of the leaf of *Aloe obliqua*. Seen from the surface, the epidermal cells appear as tolerably regular hexagons, in whose side-walls may be distinguished the primary membranes and a thick deposit of secondary layers. Among the latter, even without the application of polarized light, the innermost, rather thick layer is distinguishable by being separated from the rest by a sharp line of separation. On the application of a plate of selenite, the primary membranes, and, with the exception of the said innermost, likewise the secondary layers which lie parallel with the neutral axis of the selenite-plate, appear, according to the thickness of the plate used, red or yellow; those lying parallel with the other neutral axis green or blue; while the innermost layer exhibits, with the same regularity, the complementary colours of the other membrane lying parallel with it. The same contrast in the colour of this innermost layer and the rest of the layers, is exhibited in the transverse section of the epidermis, in which it is further observed, that this innermost layer is continuous with the unaltered cellulose membranes of the posterior inner half of the epidermal cells, and in its colour obeys the rule followed by these membranes and the subjacent parenchymatous cells.

I have demonstrated, on a former occasion*, that the chemical

* Scientific Memoirs, 2nd series, Nat. Hist. i. p. 95.

difference of the cell-membranes of the periderm and the cuticular layers of the epidermis, their incapacity to take a blue colour with iodine and sulphuric acid, and their resistance to the solvent action of sulphuric acid, do not depend upon their consisting of a constituent substance different from cellulose, but that their basis is likewise cellulose, and that this appears with its characteristic reaction with iodine, when the compound deposited in these membranes has been removed by caustic potash. Hence it required to be examined whether these membranes could be made to recover, by the same treatment, the property of acting upon polarized light in the same way as cellulose. Some experiments made with the epidermis of *Aloe obliqua* showed that this is the case completely; for even after only a few hours' maceration in solution of caustic potash, the reaction had changed perfectly into that of cellulose. Treatment of the cuticle with oxidizing agents, for instance with a solution of chromate of potash in dilute sulphuric acid, had the same effect, but less perfectly, even when the maceration in this fluid was prolonged for several days. There is no doubt, therefore, that the optical reaction of the cuticle, like its chemical reaction, is to be ascribed to the deposition of a foreign substance in its membranes composed of cellulose.

In the conversion of cotton into gun-cotton there is a change of the optical conditions analogous to that of the cellulose layers of the epidermis in their conversion into cuticular layers.

The same opposite action on polarized light to that of cellulose which is found in cuticle, is seen also in the cell-membrane of *Caulerpa* (I examined in this respect *C. prolifera*, *Freycinetii*, *clavifera*), and this not only in the tough, external, lamellated cell-membrane of the stem, the leaves, and the radical fibres (if these expressions may be used to denote the parts of a unicellular plant), but also in the substance of the branched struts which run across the cavity of the plant.

The cell-membrane of *Bryopsis*, which I examined in *B. Balbisiana* and *penicillata*, displayed an anomaly I could not explain in reference to the rest of the conditions. It consists of many concentric layers, and its substance, with the exception of the external cuticular investment, is rapidly coloured blue by iodine and chloride of zinc, like cellulose; yet only a thin external layer acted like cellulose in polarized light, and all the inner layers in the contrary way.

Finally, the starch-granules of all the plants I examined in this respect have the optical behaviour opposite to that of cellulose.

When we turn from the examination of cross-sections of cells to the elucidation of the phenomena which cells present when viewed laterally, a far greater multiformity is met with.

The longitudinal section of the cell-wall behaves to polarized light exactly like the cross-section; hence, on the application of the plate of selenite, the same colours make their appearance, according to its position. But it is far less easy to observe this phænomenon in its purity here, than in examination of cross-sections, because the preparation of delicate longitudinal sections of uniform thickness, especially of prosenchymatous tissues, is far more difficult than the cutting of good cross-sections. Too thick a slice produces colour even by itself, whereby the colours produced by the plate of selenite are rendered more or less impure. A further disturbance frequently arises out of the circumstance that the longitudinal section often takes the side-walls of the cells in an oblique direction, the sections curve round, &c., from which irregularities the colours not rarely undergo alteration, even into the complementary colours. All this renders the examination more difficult; but when good preparations are examined, the above rule will be found confirmed.

Those side-walls of cells which are viewed from the surface, and are traversed by the polarized light in a perpendicular direction, exhibit extremely diverse aspects according to the variations of their structure. To observe these phænomena in perfect purity, it is best to select such elementary organs as have their secondary membranes split into fibres,—best of all, spiral vessels. Among these, the spiral vessels of the scape of *Musa paradisiaca* afford an unsurpassable material, since they are easy to isolate, of considerable size, and regular structure. When one of these vessels, its spirals being drawn somewhat apart, is placed with its long axis in a position perpendicular to one of the Nicols, the fibres on the upper side of the vessel ascend towards the left, those of the under side towards the right, and when the selenite-plate is interposed, they exhibit the complementary colours. If the colour of one of the strata of fibres is compared with the colour of a section of a cell-wall which is placed in the same direction with the fibres, an agreement in colour is displayed. If the vessel is rotated horizontally so far that the fibres on one wall come to lie perpendicular to one of the Nicols, the colour of these fibres vanishes so far as they lie in the said direction. It is clear, therefore, that in these fibres one negative axis lies parallel with their longitudinal extension, the other perpendicular to this. It is then very easy to explain why spiral vessels which are inclined with their longitudinal axes at an angle of 45° to one of the Nicols, exhibit a totally different aspect, according as their fibre describes a more or less steeply-ascending spiral. Three cases may be distinguished. When the spiral-fibre describes a very slightly-ascending spiral, when therefore

the fibres approximate to the position of transverse fibres, in the above-mentioned position of the vessel, the fibres of the anterior and posterior walls act in the same way upon polarized light, and the vessel appears of the same colour behind and in front, like a cell clothed with cross-fibres, or like a section of a cell-wall passing at right angles to the long axis of the vessel. When the spiral vessel is so far drawn out that the fibres of its anterior and posterior walls cross at right angles, in the oblique position of the vessel above mentioned they will stand perpendicular to the two Nicols, and consequently will not act upon polarized light. When, lastly, the vessel is so much drawn out that the fibres form a very acute angle with the longitudinal axis of the vessel, they act like longitudinal fibres, and hence appear with the complementary colours of those tints which are exhibited by the fibres of a closely-wound vessel.

It need scarcely be specially mentioned that the behaviour of spiral vessels is shared by cells which contain spiral fibres, for instance the elaters of the Liverworts, the leaf-cells of *Sphagnum*, the cells of the sporangium of *Equisetum Telmateia*, the elaters of the same plant, the spiral cells in the stem and petiole of *Nepenthes*, those of the Orchidæ, &c.

Completely analogous conditions are observed in those elementary organs whose secondary fibres form a more or less regular network, as in the reticulated vessels of the Monocotyledons, the scalariform ducts of the Ferns, the reticulated parenchyma-cells of the wing of the seed of *Swietenia Mahogani*, &c. Of course the fibres here do not agree in colour in any position of the cell, but exhibit, according to their varied positions, the same colours as the cell-walls in the section of a parenchymatous cellular tissue.

As is well known, we find transitions from those cells which have the secondary layers divided into separate fibres, to the apparently homogeneous cell-membranes, or structures of that kind where the secondary layers are not composed of separable fibres, yet possess a fibrous structure, indicated by a finer or coarser striation, and are more easily torn in the direction of these streaks than in any other direction. This condition gave rise to the notorious dispute whether or not cell-membrane is composed of primitive fibres.

Among unpitted cells with fibre-like striation of the membrane, many Confervæ, for example *C. Melagonium*, and further, the *Cladophoræ*, are well known to be remarkable for the circumstance that they exhibit two systems of such streaks, crossing at right angles. It was a question here whether one alone of these systems determined the optical character of the membrane, or the two exerted an equal and opposite effect, mutually acu-

tralizing their action, as occurs in two cross plates of mica. Observation shows that the former is the case, and that the colour which the membrane exhibits on the application of a plate of selenite depends on the direction of the longitudinal striation. The direction of this striation is not the same in all *Cladophoræ*. In some, as in *C. glomerata* and *longissima*, one system of striæ, determining the optical conditions, runs parallel with the long axis of the cell; hence the cell-membrane of these species appears colourless when the cell is placed perpendicular to the Nicols, and in an oblique position exhibits the same colours as a section through a cell-wall lying parallel with the cell. In other species, as in *C. hospita*, the longitudinal striæ run in the direction of a steeply-ascending spiral, and the action of the light is altered accordingly.

In an analogous way, the cell-membranes of the Characeæ also exhibit two systems of streaks, crossing at right angles. But the striation is different from that of the *Cladophoræ*. It does not present, as in them, uniform and parallel lines, but the cells appear traversed in a transverse direction by fine streaks, which form a network with long meshes, while thicker bundles of fibres run longitudinally, often exhibiting an undulating course. This condition occurs both in the central cell of *Chara*, for example in *C. equisetina*, Kütz., and in the cell-wall of *Nitella*, for instance in *N. flexilis*, *mucronata*, *fasciculata*, and *syncarpa*. These membranes act very powerfully upon polarized light, but, what is remarkable, they exhibit in the same positions the opposite colours to those of the *Cladophoræ*. This might give rise to the conjecture, that the *Charæ* form, in regard to the optical conditions of their cell-membranes, similar exceptions to the general rule to those in *Caulerpa*; but the examination of transverse sections of *Nitella fasciculata* and *Chara equisetina* did not confirm this, for these behaved exactly like the transverse section of an ordinary cell. Since, then, on the lateral view of the cell the colouring is the opposite of that of the cell-membrane of *Cladophora*, and agrees with that of a spiral vessel with a very gently-ascending fibre, it is clear that in the *Charæ* the optical character of the membrane is determined by the transverse fibres.

Phænomena exactly analogous to those of cells in which the membrane has visible fibre-like streaks, are exhibited by cells in which only traces of this appearance are to be detected, or whose membrane appears to be completely homogeneous; since their membrane, according to the direction in relation to the Nicol, is sometimes invisible, sometimes more or less brightly illuminated, and, on the application of the selenite-plate, develops a yellow or blue colour. Here exists, on the one hand, evidence that the

apparently homogeneous cell-membrane is not really so; and on the other hand, the position in which the cell must be placed in order to be visible, and the colour produced by the selenite-plate, enable us to ascertain the direction of its invisible fibrillation. In this respect, for example, isolated vessels from the trunks of Tree-ferns are very instructive: on their membranes occur largish spots, smooth and perfectly homogeneous, corresponding to the angles of adjacent cells; on the application of the selenite-plate, these exhibit the same colour as the fibres running between the pits, and hence demonstrate that the entire membrane has a fibrous structure in the transverse direction. Beautiful examples of this are furnished also by the wood-cells of the Coniferæ and Cycadææ, in which, very frequently, even when a definite fibrillation is not indicated by striation of the membrane with the ordinary microscope, the direction in which the fibres* run may be determined by the angle at which the slits of the pits stand obliquely to the long axis. This angle amounts, for instance in the wood-cells of a *Cycas*, to nearly 45° . If the cells are placed perpendicularly to one Nicol, these membranes appear, according as the fibres are directed to the right or left, either blue or yellow; on the other hand, they become colourless when the cell is inclined at an angle of 45° to the Nicol, while in this position the illumination and brilliancy of colour of the side-walls standing perpendicular to the surface of the object-slider attain their maximum. An analogous behaviour is exhibited by the cells of *Torreya taxifolia*, whose fibres ascend at an angle of 70° , and the cells of Fir-wood, with an angle of 68° ,—only these, for reasons readily explained, did not show the greatest brilliancy of colour when the cell was placed perpendicular to a Nicol, but when it was so far inclined that its fibres formed an angle of 45° with the Nicol.

Peculiar phænomena are exhibited by the lateral borders of a cell containing an oblique or transverse fibre on its walls. The longitudinal section of a cell-wall behaves to polarized light, as above noticed, like a transverse section of the same; and the same holds good, in spiral vessels, annular vessels, scalariform ducts, &c., of the side borders at which the fibres of the anterior side curve downwards to reach the posterior side. In consequence of this, the fibres present, at the places of curvature, sometimes the same, sometimes the opposite colour from that of the fibres running on the horizontally-lying lateral surfaces, according as the latter ascend in a more or less steep spiral.

* When I speak here and in other places of *fibres*, I do it for the sake of brevity of expression, and by no means thereby intend to defend the doctrine of the existence of primitive fibres.

When, for example, a spiral vessel with a gently-ascending spiral is inclined at an angle of 45° to one of the Nicols, and thus the fibres of its posterior and anterior sides react in the same way on polarized light, in the manner of transverse fibres, appearing of a yellow colour, the places of curvature of the same will be blue, thus appearing of the same colour as if the lateral walls were composed of longitudinal fibres. The places of curvature appear of the same colours if the fibres of the anterior and posterior sides ascend at an angle of 45° to the longitudinal axis of the vessel, and in consequence of this, in the said position of the vessel remain uncoloured. When, on the other hand, the fibres describe a very steeply-ascending spiral, and the fibres lying on the posterior and anterior sides of the vessel thus act in the manner of longitudinal fibres, their colour agrees with that of the places of curvature. Constant and regular as these phænomena appear when we examine organs of very regular structure, like spiral vessels, in examining thick-walled cylindrical cells, for instance the hairs of *Boragineæ*, or isolated prismatic cells, such as wood- and liber-cells, many exceptions will be found, partly because the latter are subject to much irregularity in the course of the fibres from the oblique, variously changing inclinations of the side-walls of the cells, partly because, as it appears, the direction of the fibres is not always the same in the different layers of one and the same cell, so that, for instance, it describes a less steep spiral in the outer lamellæ of the cell-wall than in the inner.

The phænomena which the cell-walls exhibit in polarized light undergo, again, manifold modifications when two cell-membranes with differently directed fibres lie immediately one over the other. This naturally occurs with extreme frequency in the cellular tissue of plants when the fibrillation of the cells follows a spiral direction, since in that case the fibres will pursue opposite directions on the coherent membranes of two adjacent cells. When two such membranes are viewed lying one over the other in a horizontal direction, the lower one, as a doubly-refractive substance, must exert upon the light coming from the Nicol an analogous although weaker influence to that of a plate of selenite, and the effect of this must be more or less clearly indicated in the phænomena presented by the upper membrane. An influence of this kind must likewise make itself felt even when the membranes do not lie immediately one on the other, but are separated by a greater or less space, as for example the upper and lower membranes of a cell by the cell-cavity. Here, again, the spiral vessels of *Musa* may be used as a suitable object, in which these phænomena may be observed with certainty.

If we examine a vessel, placed perpendicular to one of the

Nicols, which has suffered no compression, and which is so far drawn out that the curves of the upper and under sides stand about at right angles to each other, the greater part of the turns are freely exposed to the light coming from the lower Nicol, and the two layers of fibres only lie one over the other in triangular spaces at the points of curvature of the fibrous bands at the margins of the vessel. In such a vessel, as above remarked, when a plate of selenite is interposed, the oppositely wound fibres of the upper and lower sides appear in complementary colours. These colours present themselves in great purity in all parts where the fibres lie free, but appear dulled in the triangles above noticed. It here depends solely upon the focussing of the microscope which colour we produce in these crossing-places: if we focus carefully to the fibre of the upper wall, its colour (no matter whether yellow or blue) will be almost pure, and little affected by the colouring of the lower stratum of fibres, lying under these circumstances out of focus; on the other hand, if we focus through the upper stratum of fibres to the lower, this appears with its proper colour, without the upper stratum of fibres, which cannot now produce any defined image on the retina, producing any very great disturbance. Properly the colours of the two layers of fibres should completely destroy each other, and it cannot be doubted the light has undergone a modification in passing through the inferior layer of fibres, and again suffers it in the upper layer; but with the isolated position of the fibres produced by the longitudinal stretching of the vessel, this light forms but an inconsiderable portion of the total mass of light coming from the plate of selenite, and remains almost ineffective after traversing the path from the lower to the upper layer of fibres, so that no very striking modification of the colouring of the upper layer of fibres can make itself felt.

On the other hand, matters are totally changed when the vessel is pressed flat in the compressor, so that the fibres of the two layers come to lie one immediately above another at the crossing-points, where the light modified by the lower fibre then penetrates immediately into the upper fibre, without mixing with the light penetrating unaltered beside the fibres of the lower layer. Here the action of the two layers of fibres upon one another is in most cases most evident, but it is indicated in very various ways, according as the crossing of the fibres occurs or not at a right angle.

If the fibres cross at right angles, the effect upon polarized light exerted by the lower stratum of fibres is removed by the upper stratum, in the same way as happens with two plates of mica crossing at right angles; hence the fibres should be invisible at the crossing-points. Now, this does not, it is true,

occur quite strictly; but when the Nicols are used alone, the crossing-points appear more or less black, while the rest of the fibres appear white; and when the selenite-plate is interposed, the crossing-points exhibit the colour of the field, while the uncrossed portions of the fibres are coloured yellow or blue, according to their position. For reasons readily perceived, the want of colour at the crossing-points remains the same, however the preparation may be rotated in a horizontal direction.

But when the superposed fibres do not cross at right angles (no matter whether more acutely or more obtusely), the phenomena exhibited at the crossing-points are essentially different. When a vessel of such kind stands perpendicular to one of the Nicols, the right-ascending spiral will appear in the complementary colour of the left-ascending spiral, as in an uncompressed vessel, so far as the fibres do not overlies; but at the crossing-points the lower stratum of fibres shows through the upper with its proper colour (no matter whether blue or yellow) almost unaltered, while the upper stratum is scarcely seen. When, however, such a vessel is placed at an angle of 45° to the Nicol, when the layers of fibres appear of the same colour, the effects of the two fibres are added together at the crossing-points, and at these places we find an analogous, but brighter, colour than that of the free part of the fibres,—bright yellow instead of dull yellow, bright blue instead of dull blue.

In the spiral vessels prepared in the above manner, the fibres of the upper and under sides act with equal force upon polarized light on account of their equal thickness, and the course of the fibres is perfectly regular: an equal regularity of the phenomena there presented will not easily be found in the examination of two adherent walls of adjacent cells, because here the unequal thickness of the cell-wall and the irregular course of the fibres diverted by the canals of the pits, produce disturbances, whence the colouring of the cell-walls frequently becomes unequal, and even complementary colours are found in neighbouring parts. This is the case in a high degree in thick-walled cells of somewhat irregular form, as in liber-cells.

Finally, it must be mentioned, in reference to the cell-wall penetrated in a perpendicular direction by polarized light, that in many cases, as already noticed by Schacht, the vicinity of a round pit is distinguished by a black cross similar to that exhibited by the cross-section of cylindrical cells. This cross is most strikingly seen in the border which surrounds the pit of Fir-wood; it occurs, less sharply defined externally, on the pits of the endosperm of *Phytelephas*, of Palms, and on many wood-cells. The origin of this cross is easily explicable, from the fact that the fibres of the membrane are diverted round the pits in

circular curves, to which is added, in the pits of Fir-wood, the circumstance that the cell-wall is protruded inwards into the cell, in a globular form, over the cavity which forms the border of the pit.

Passing from the cell-wall to the cell-contents, we find that the behaviour of starch-granules with polarized light has already been investigated by so many persons, that scarcely any notice will be required respecting it. It is universally known that they show a black cross, like the transverse section of a cylindrical cell; that the point from which the arms of the cross run out always coincides with the organic centre of the granule, with the so-called hilum; that the arms of the cross stand perpendicular to the lamellæ of the granule, and that therefore the cross is often exceedingly irregular*, from the excentric arrangement so frequently presented by the lamellæ (most strikingly in the starch-granules of the tubers of *Canna indica*, of Galanga-root, of the milky juice of *Euphorbia*, &c.). I have likewise explained above, that the colours which the starch-granules exhibit on the application of a plate of selenite, are *positive*, and opposite to those of cellulose. The swelling-up of starch-granules in boiling water, strong acids, or caustic alkalies, removes from their substance the power of acting upon polarized light (at least in a degree capable of detection with our instruments). It offers a very attractive spectacle to observe this process in the starch of the Potato with the application of a selenite-plate. When a drop of solution of potash is added to the water in which the granules lie, the latter swell from without inwards: as far as their substance remains yet unattacked, it exhibits the most vivid colours; the swollen portion is quite sharply separated from the still hard parts, and during its expansion it exhibits pale colours, which vanish in the completely gelatinized parts.

In my opinion, inulin does not occur in the form of granules in the cells of living plants, but in solution. In the parenchyma-cells of dried roots of *Inula Helenium*, it presented itself in the form of irregular angular masses, not expanding in cold water, acting strongly upon polarized light, which possibly may depend simply upon mechanical tension. It was precipitated from the boiling solution in the form of small, irregular, roundish lumps, which acted only weakly and in an irregular manner upon polarized light.

I could not observe any effect upon polarized light in chlorophyll-granules or the chlorophyll-bands of *Spirogyra*. The starch-granules contained in them displayed the ordinary phæ-

* That particular observers, for example Pereira, did not see the black cross in the starch-granules of certain plants, for instance in the seeds of wheat and rice, is attributable to the imperfection of the instruments with which they observed.

nomena; but the green colouring matter is in a high degree obstructive to the development of a bright light, as is shown by the comparison of the chlorophyll of a leaf bleached by alcohol with a fresh leaf of the same plant.

In the granules of oleaginous seeds (Hartig's *aleurion-granules*) I could usually find no sign of double refraction; distinct traces were, however, displayed, on the application of a plate of mica, in the seed of *Attalea funifera*.

In the primordial utricle I ordinarily found no trace of double refraction. In the *Spirogyra*, however, it exhibited, after contraction with weak alcohol, on the application of a plate of selenite or mica, a very weak but quite evident reaction like that of cellulose.

My observations on the last three structures are, as is evident, very insufficient; further improvements of the observing instruments are required in order to arrive at decided results respecting their behaviour with polarized light.

The polarizing microscope, even in its present condition, is excellently adapted for the discovery of crystals in plants, since these appear with surprising brilliancy on the dark field. Although it has been long known that crystals are very widely diffused formations in the vegetable kingdom, and that scarcely a plant exists, in the higher orders, in which they may not be discovered,—yet one is surprised, on the application of polarized light, to find the crystals far more frequent, and in far greater quantity, than one is accustomed to see on investigation with the ordinary microscope. The quantity of them in many Lichens, for instance in *Lecanora tartarea*, is quite surprising in amount; and they will likewise be found in the tissue of many embryos, where they would otherwise easily escape notice on account of their small size and the granular contents of the cells; but even in other parts where they have long been known and are more readily discoverable, they may be detected more easily and in greater quantity, and as crystals, in polarized light. I will mention, in reference to this, only the stellate hairs of the air-canal of *Nymphæa*, in each nodule of which lies a crystal, soluble in strong acid. The crystals mostly appear, even without the application of a selenite-plate, in brilliant colours; and there is not a more attractive spectacle than the view of a great quantity of [sulphate of lime?] crystals in the petiole of many *Musaceæ*, e. g. *Urania speciosa*, or of the larger raphides, such as are so common for instance in the tissues of the Aloes. Whether crystals occur which belong to the regular system, and consequently do not act upon polarized light, is unknown to me, but I have met with none hitherto.—(A. H.)

Tübingen, November 1857.